Mutations in components of antiviral or microbial defense as a basis for breast cancer

Bernard Friedenson

Dept. of Biochemistry and Molecular Genetics
College of Medicine
University of Illinois Chicago
Correspondence to: Bernard Friedenson
email: molmeddoc@yahoo.com
Telephone/Fax 847-298-1641
In-depth functional analyses of thousands of breast cancer gene mutations reveals vastly different sets of mutated genes in each of 21 different breast cancer genomes. Despite differences in which genes are mutated, innate immunity pathways and metabolic reactions supporting them are always damaged. These functions depend on many different genes. Mutations may be rare individually but each set of mutations affects some aspect of pathogen recognition and defense, especially those involving viruses. Some mutations cause a dysregulated immune response, which can also increase cancer risks. The frequency of an individual mutation may be less important than its effect on function. This work demonstrates that acquired immune deficiencies and dysregulation in cancer can occur because of mutations. Abnormal immune responses represent a hidden variable in breast cancer - viral association studies. Compensating for these abnormalities may open many new opportunities for cancer prevention and therapy.

**Keywords**
Breast cancer infection, breast cancer immunity, breast cancer virus, genome biochemistry
Introduction

An in-depth analysis of thousands of breast cancer gene mutations in 21 different breast cancer genomes may provide a clearer view of how cancer originates. The analysis revealed vastly different sets of mutated genes in every breast cancer with only occasional genes mutated in more than one breast cancer. Immune and antiviral functions protecting against pathogens are complex, requiring many genes dispersed throughout the genome. Mutations in many different genes can disable some aspect of immunity or other defenses, causing acquired immunodeficiency states and increase susceptibility to cancer. Some mutations cause a dysregulated immune response, which also increases cancer risks. Mutations in a specific gene may occur more than once but the frequency of an individual mutation may be less important than its effect on function. This view might permit more effective approaches to prevention and therapy because it suggests compensating for or exploiting functional deficits as distinct from trying to exploit individual specific abnormalities in a few shared driver genes.

As causes of breast cancer, some studies implicate tumor viruses such as a human form of mouse mammary tumor virus (HMTV), Epstein Barr virus (EBV) or human papilloma virus (HPV) (Glenn et al. 2012). HMTV, for example, occurs in human mammary epithelium; antibodies have been reported; HMTV-breast cancer associations correlate with geographical prevalence of mice; viral particles have been isolated from primary cultures of human breast cancer cells; 5% of breast milk samples contain viral sequences; and MMTV can infect human mammary epithelial cells (Holland and Pogo 2012). Mouse mammary tumor virus causes breast cancer in mice.

But no single virus is always found in breast cancer and the idea that a virus causes breast cancer remains controversial. Despite well-done experiments, exactly opposite results from different groups suggest “lurking variables” are not being considered. One such additional variable is the condition of the immune response.

The immune response to pathogens utilizes both immediate (innate) and delayed (adaptive or induced) immune responses. Because many pathogens can multiply quickly, a rapid innate response is essential to allow time until a more specific and effective adaptive immune response can develop. The mammary gland probably evolved from the innate immune system (Vorbach et al. 2006). Conserved signaling pathways are involved in immunity as well as in the development and function of the mammary gland. Lactation, as constituted today, evolved as an inflammatory response to tissue damage and infection and inflammatory molecules became key regulators (Vorbach et al. 2006).
A host derived immune response probably always accompanies human breast cancer. Breast cancers are heavily infiltrated with cells from the immune system within the tumor or the stroma. Among 3403 cases, CD8+ tumor infiltrating lymphocytes were in the tumor in 32% and in the stroma in 61% of the cases (Liu et al. 2012). Lymphoid infiltrates are found in about half the prophylactic mastectomies from BRCA2 mutation carriers, who have a high risk of breast cancer. Results of studying such surgically removed breast tissues clearly show immune responses occur even before cancer can be detected (Hermsen et al. 2005).

There are different kinds of immune cells within infiltrates in breast cancers and their stroma. These immune responses are unable to prevent or clear breast cancer. This failure may be because mutations impair the immune response or cause its dysregulation so that it can even potentiate cancer. There is a general immune system dysfunction in breast cancer patients with impaired production of interferon gamma and other cytokines (Campbell et al. 2005). Alterations in chemokine levels are associated with cancer progression (Porter et al. 2003). NK cell activity from the innate immune system was significantly lower in individuals with strong family histories of breast cancer vs. individuals without this history (Cunningham-Rundles et al. 1981). Even early stage breast cancer patients lose immune functions (Poschke et al. 2012). In breast cancer patients, regulatory T-cells in malignant lesions curtail other T-cell and NK cell responses (Curigliano 2011). Common genetic variants in innate immunity genes are strongly associated with breast cancer risk in Korean women (Lee et al. 2009). Defects in pathways mediated by BRCA1 and BRCA2 “breast cancer genes” are associated with cancers involving the immune system (Friedenson 2007) and impaired immunity. Variants of breast cancer cell lines are aggressively metastatic in immunodeficient mice. Suppression of an innate immune pathway intrinsic to breast cancer cells restricts immune surveillance to enable metastasis (Bidwell et al. 2012)

Innate immune sensors that can recognize foreign nucleic acids from viral infections exist in breast and in most other cells. These sensors such as the Toll-like receptors (TLRs) then induce cellular and molecular effectors with broad antiviral activity and promote inflammation to increase potency of adaptive immune responses. All ten known TLRs have been found in breast cancers and in breast milk. Specifically, 60 tumor samples (80% of those tested) were positive for TLR5. TLR5 was highly expressed in most invasive ductal carcinomas (IDC) and moderately expressed in medullary carcinomas and invasive lobular carcinoma (Cai et al. 2011b). Retinoic acid can induce the intrinsic ability of breast cancer cells to recognize double-stranded RNA through the up regulation
of TLR3 expression (Bernardo et al. 2013). TLR4 expression in 50 primary invasive ductal carcinoma and 17 lymph node sections was significantly associated with local metastasis and tumor grade (Ehsan et al. 2013). Low tumor TLR9 expression is associated with significantly shortened disease-specific survival in patients with triple negative but not with ER+ breast cancers (Tuomela et al. 2012). Members of the TLR9 subfamily are RNA (TLRs 3, 7, and 8) and DNA (TLR9) receptors.

Additional innate immune sensors include the RIG-I helicase family, cytosolic helicases that primarily sense viral RNA. NLRP3, one of the cytoplasmic nucleotide oligomerization domain (NOD) -like receptors, can also recognize viral RNA and DNA. Scavenger receptors can modulate infectivity of Hepatitis C virus. Other proteins with antiviral activity have not been placed in the innate or adaptive immune system.

The recent availability of breast cancer genomic sequences permits considering the possibility that breast cancers develop because of varying losses in protective immune function or because of its dysregulation. These abnormalities could represent a significant variable in virus-breast cancer association studies but an abnormal immune response could contribute to breast cancer whatever its cause. Publicly available data from 21 different breast cancer genomes was researched in depth looking for mutations, deletions, and rearrangement of genes essential for breast immune responses.

**Abnormalities in genes required for innate immunity and its regulation in breast cancer cells.** Figs. 1-2 are examples that outline steps in pathways where mutations have damaged defenses against RNA and DNA tumor viruses, respectively. Table 1 documents examples of breast cancer mutations affecting immune response functions and/or their regulation. Tables 1-2 also includes mutations not shown in Figs 1-2 expected to affect immune responses. Multiple components of immune defenses are mutated in every one of 21 breast cancer genomes. Mutations also dysregulate the immune response (Table 1) and may also contribute to cancer i.e. by causing chronic inflammation. The mutations are indicated in the Figures as affecting processes such as cytokine production, autophagy, etc. rather than as affecting specific genes. Breast cancer mutations always involved these common functions or their regulation. The affected functions depend on many genes dispersed throughout the genome so most genes are only mutated in one cancer. Mutations in the same gene are uncommon and each breast cancer genome has a different set of these mutations.

Antigen recognition, signaling essential to transmit the immune responses, and immune regulation can all be damaged. Large differences exist in distributions and numbers of mutations among the breast cancer genomes. There are also large differences
in the numbers of DNA breaks, and non-coding mutations (Nik-Zainal et al. 2012). Despite these differences, major common properties of many mutations is that they cripple some aspect of innate immunity, its regulation, or its connections to adaptive immunity. Damage to some gene products shown in Figs. 1-2 can potentially dysregulate immunity and inflammation in breast cancers (Table 1), damaging specific responses to antigens or to pathogens. Dysregulation could conceivably result from mutations alone without exposure to antigens or pathogens, impairing host response to breast cancer whatever its cause. For example, in breast cancer PD3905a, mutation occurs in IL1R2 within the interleukin receptor gene cluster. Inactivity of IL-1R2 generates an inflammatory necrotic phenotype (Zheng et al. 2013). In breast cancers PD3905a, PD4103a, and PD4116a regulators of autophagy and lysosomal trafficking are damaged. In PD4107a, mutation occurs in the IFNGR1 gene, which has crucial roles in regulating both innate and adaptive immunity. In breast cancers PD4103a and PD4120a, GATA3, a critical early regulator of innate lymphoid cell development is mutated. Many breast cancer mutations cause defects in antiviral defenses, increasing risks for viral infections in the breast.

Examples of damage to specific genes encoding antiviral defenses in different breast cancers. During infection by RNA viruses, single- and double-stranded RNA are recognized by the host and induce innate immune responses by several proposed mechanisms (Fig. 1). Viral products such as 5'-PPP-RNA represent a foreign molecular signature that distinguishes it from normal host nucleic acids. Host proteins responsible for sensing or interacting with such foreign nucleic acids include the TLR’s present in the breast. IFITs (interferon-induced protein with tetrapricopeptide repeats) are among the most potently expressed proteins of a group of interferon-stimulated genes, which result from virally triggered signaling pathways that lead to the production of interferons and other cytokines (Abbas et al. 2013). IFITs directly engage PPP-RNA and a group of them is deleted in breast cancer PD3904a (Table 1).

The cellular enzyme ADAR (adenosine deaminase acting on RNA) is stimulated by interferon in virally infected cells. Normally ADAR leads to the presence of inosine-containing RNA. Inosine RNA may then further stimulate breast innate immunity. The ADAR gene is involved in an inter-chromosomal rearrangement in breast cancer PD4115a (Table 1). A-to-I editing encoded by ADAR can lead to either enhanced or reduced virus growth and persistence depending upon the specific virus (Samuel 2011). ADAR-1 causes hypermutation of some RNA viruses such as HTLV-2 and STLV-3(Ko et
The AID/APOBEC family of 7 proteins constitutes another innate antiretroviral defense system based on RNA editing. APOBEC4 is mutated in breast cancer PD4109.

In Fig. 2, these recognition functions are extended to the recognition of viral RNA produced by transcription after DNA virus infections. In Fig. 2, PD3905a is shown as having a defect in detecting viral DNA. In PD3905a, the histone deacetylase 7 (HDAC7) gene is mutated. HDAC 7 was the most readily detected class II HDAC in an EBV producer and a non-producer cell line, suggesting involvement in repression during EBV latency (Bryant and Farrell 2002).

The presence of DNA anywhere other than the nucleus or mitochondria is perceived by the innate immune system as a Damage-Associated Molecular Pattern (DAMP) and triggers the assembly of an AIM2 inflammasome as a receptor for cytoplasmic DNA (Fig. 2). The AIM2 inflammasome activates inflammation and promotes maturation of inflammatory cytokines. AIM2 is mutated in breast cancer PD4086a but normal in other breast cancers. Fig. 2 also shows potential indirect actions of other mutations such as DSTYK (a caspase inducer) and CIQTNF1 (a target of the inflammatory response mediator IIL1β, Fig. 2 and Table 1).

**Autophagy.** Figs. 1-2 show 7 breast cancers with mutations in genes encoding or regulating autophagy functions (e.g. PD3851a, PD3904a, PD3905a, PD4103a, PD4107a, PD4116a, and PD4120a). Autophagy ("self-eating") is a degradation process, in which abnormal cytoplasmic content is engulfed and degraded by the lysosome. Autophagy is also a notable pathway mediating innate immune responses that eliminate pathogens. A subset of viruses and bacteria subvert autophagic pathways to promote their own replication. The importance of autophagy in the cellular response is illustrated by the diversity of viruses — including HIV-1, influenza and herpes viruses — that can inhibit autophagy (Garcia-Sastre 2013).

**NF-kB responses.** Nine breast cancers are shown with mutations affecting NF-kB signaling or its regulation (Figs. 1 - 2). Because of its extensive role in immune action, NF-kB has been termed the central mediator of the immune response. Mutations in NF-kB pathways or their regulation may affect host response to breast cancer whatever its cause. Transcriptional regulators of the NF-kB/IκB family promote the expression of hundreds of target genes, markedly affecting the host immune response and cell cycle. These proteins include many cytokines and chemokines, receptors required for immune recognition, proteins involved in antigen presentation, adhesion receptors involved in transmigration across blood vessels walls, growth factors and proto-oncogenes. Relevant
inducers are typically viruses, or other activators or mediators of innate immunity (Hiscott et al. 2001).

NF-kB regulation is influenced by many different genes and is abnormal in some breast cancers (Figs. 1-2). For example, PD3904a has a mutation in RHEBL1 (Yuan et al. 2005), a positive regulator of NF-kB signaling (Figs 1-2 and Table 1). Even mutations in other pathways can impact the activation of NF-kB. PD3890a and PD3904a have mutations within the NFAT pathway. The NFAT family of transcription factors regulates cytokine gene expression by binding to the promoter/enhancer regions of antigen-responsive genes, usually in cooperation with heterologous DNA-binding partners. In some systems, activation of NFAT and NF-kB are mutually dependent and even bind at the same promoter (Cai et al. 2011a; Fu et al. 2006).

Mutations in proinflammatory NF-kB pathways can also contribute to human viral oncogenic transformation. Viral oncogene products, including human T-cell leukemia virus type 1 (HTLV-1) Tax protein and EBV latent infection membrane protein 1, each act by distinct mechanisms to disrupt NF-kB regulation and initiate viral transformation (Hiscott et al. 2001). Localization of NF-kB encoding genes at sites of chromosomal translocations and genomic rearrangements in cancer, high levels of NF-kB activity in many breast cancer cells, and constitutive nuclear NF-kB complexes in virally mediated Hodgkin’s lymphoma cells all support viral participation.

Inflammatory cytokines and interferons. At least ten breast cancers show damage to genes essential for inflammatory cytokines (Figs. 1-2, lower left) but this can occur in different ways. Damage to interferon inducible genes can also occur at multiple points but the result is to disable some aspect of interferon mediated protection (Figs 1-2, lower right) or to change inflammatory processes, required for a proper adaptive immune response.

In the breast cancer PD4107a genome, mutation of a single gene may affect several relevant functions at once. PPP2R1A gene mutations can deregulate NF-kB activation and IL-2 or IFN-γ production (Eitelhuber et al. 2011). PD4107a also has a deletion in IL20RA and IL22RA2 which disables JAK-STAT signal transmission of interferon activities (Table 1). Further illustrations of how mutations affect cytokine or interferon signaling are documented in Table 1.

Colony stimulating factors. Colony-Stimulating Factor-1 (CSF-1) and its receptor (CSF-1R) regulate macrophage production and mammary gland development. CSFs are in clinical trials as treatment for some types of cancer. CSFs can increase the
production of complement components. Complement components are mutated in a few of the breast cancer genomes. In the 21 breast cancers here, CSFs genes were normal but mutations in other genes could affect CSF expression. A tandem duplication in breast cancer PD4006a included YY2, a significant regulator of CSF3. In contrast, HAX1 is essential for CSF-triggered granulopoiesis and a genetic defect in HAX1 causes premature apoptosis of myeloid progenitor cells. HAX1 is deleted in breast cancer PD4109a.

Other mechanisms of interfering with the innate immune response not shown in Figs. 1 and 2.

Figs. 1-2 are based on basic outline models of innate immune response pathways (Mogensen 2009; Barber 2011) but many specific steps are omitted for clarity. The omitted specific steps provide further opportunities for mutations in additional genes to interfere with the immune response (e.g. DSTYK and CIQTNFI in Fig 2). These mutations may affect response to other pathogens, other innate immunity receptors or pathways, damage connections between innate and adaptive immunity or dysregulate the response.

Examples of mutation in other pathogen associated molecular patterns (PAMPs). Receptors for pathogen-associated molecular patterns are pattern recognition receptor proteins that occur outside the cell, in the cytoplasm, or in the nucleus. PAMPs can be associated with microbial pathogens or cellular stress.

In breast cancers PD3904a and PD4120a, the gene encoding a pattern recognition scavenger receptor CD163L1 is mutated (Table 1). Generally this may encourage further mutations because scavenger receptors are essential to remove waste or harmful substances. Cysteine-rich scavenger receptors can serve as pattern recognition receptors for bacteria and for hepatitis C virus (69). The DSCAM gene, involved in a rearrangement in PD4088a (Table 1) encodes a pattern recognition receptor that can produce thousands of isoforms via mutually exclusive alternative splicing, i.e. a cloud of different immune effectors with different specificities (Ziauddin and Schneider 2012).

Breast cancer gene mutations can interfere with connections to adaptive immunity. PD4194a contains a mutation in the gene encoding E-cadherin / CDH1 (Table 1). A common feature shared between several putative human cancer-associated viruses, such as EBV, HBV, HCVs, and HPV is the ability to reduce the expression of cellular E-cadherin. Of the common tumor viruses, HPV16 is highly efficient at reducing E-cadherin levels. Mutation of the E-cadherin gene may affect the efficiency of the immune response (Laurson et al. 2010) but E-cadherin is not shown in Figs. 1-2.
ARID2 is mutated in the BRCA1-associated breast cancer PD3905. ARID2 encodes a part of a chromatin remodeling complex and is not indicated in Figs. 1-2. Infected host cells harboring ARID2 mutations may have lost their ability to express higher levels of class I MHC molecules in response to IFN, making them less visible to cytotoxic T lymphocytes (Zhao et al. 2011; Li et al. 2011).

Mutations in genes encoding unclassified proteins related to viral infections: Selection for or against different infections by breast gene mutations. Because different viruses require different sets of host cellular genes in order to infect and proliferate, mutation of cellular genes in breast cancers can alter targets that affect susceptibility to viral infections. Some of these targets are not clearly linked to innate immunity. In breast cancer PD3905a, SLC48A8 is mutated. SLC48A8 mRNA levels are decreased by antisense transcripts from LTR promoter elements of human endogenous retroviruses. In PD4107a mutation of may protect against entry of several α herpesviruses, because the PVRL1 protein mediates their entry.

There are many similarities, but surface proteins from different viruses can induce differences in host chemokines and cytokines (Mogensen and Paludan 2001). The secreted protein TGF-β2 (deleted in PD4088a), has a major effect on IgA production from adjacent plasma cells. Deletion of ATP7IP makes EBV lytic activation unlikely (Chang et al. 2005), but ARHGDIB is a lymphoid-specific intrinsic negative regulator of HIV-1 replication (Watanabe et al. 2012). Similarly desmoglein 2 (DSG2) acts as a high affinity receptor for human adenoviruses, so DSG2 mutation in PD4120a would argue against adenovirus infections.

Tandem Duplications can favor or suppress some infections

In the PD4006a breast cancer, a tandem duplication on chromosome 17 from bases 44,093,205 to 46,176,285 includes MIR152. MIR152 overexpression inhibited production of cytokines, and may participate in fine tuning the innate immune response (Liu et al. 2010). In breast cancer PD3945a, a tandem duplication of about 1 million bases on chromosome 2 (positions 69,554,984 to 70,626,823) could theoretically alter susceptibility to some infections. MXD1 is also encoded in the duplicated region and MXD1 protein product can participate in repressing EBV latent membrane protein 1 (Jansson et al. 2007) MXD1 also antagonizes the effects of c-Myc, a transcription factor whose activity is frequently elevated in cancer. Myc can impose a non-immunogenic phenotype in virally infected cells (Staege et al. 2002).
In the PD3851a breast cancer, a tandem duplication contains genes that may represent bolstered immune defenses. For example, the ZBP1 gene product suppresses HSV-1 growth. This suppression is independent of DNA sensing through mechanisms involving suppression of viral genomes (Pham et al. 2013). ZBP1 also has other activities. ZBP1 decreases cancer cell migration and metastatic proliferation. In breast cancer PD4086a, an intrachromosomal tandem duplication involves the PTPN1 gene. PTPN1 negatively regulates MyD88 and TRIF dependent proinflammatory cytokine and type I IFN production (Figs 1-2).

Many other tandem duplications only involve part of a gene sequence involved in immunity. This may alter the regulation, tissue specific expression or affect functions.

**Independent Supporting Evidence**

In at least five different sporadic breast cancers with normal BRCA1 and BRCA2 genes, there are mutations related to pathways depending on BRCA1/2 gene products (PD4088a, PD4103a, PD4109a, PD4120a, and PD4198a). Mutations of genes encoding proteins within this pathway are independently known to favor some viral infections. HPV proteins E6 and E7 interact with BRCA1 and BRCA1 and BRCA2 gene mutations increase risk for cervical cancer (caused by HPV’s) (Zhang et al. 2005; Friedenson 2010b, c). Other related gene mutations cause hereditary conditions with proven increases in susceptibility to specific sets of infections and cancers. Three connections are specific to breast cancers with mutations in the DNA damage response pathway dependent on BRCA1 and BRCA2. Two of these mutations are found in PD4103a (e.g. FANCB and NBN)(Lowy and Gillison 2003; Spardy et al. 2007; Gregorek et al. 2010). PD4198a shows a different connection because it contains an ATM mutation. ATM participates in the DNA damage response affecting both the immune system and breast cancer risk. The DNA damage response may also participate in alerting the immune system to the presence of potentially dangerous cells (Gasser et al. 2005)

**Acquired immunodeficiency states reactivate or stimulate known cancer viruses.** Immune system dysfunctions that favor infections are well known. Suppression of the immune system before kidney transplantation increases risks for breast and three other cancers (Hibberd et al. 2013). Recipients have a well-documented increased incidence of human HPV related malignancies. Nearly 50% of male kidney transplant recipients were positive for HPV sequences, most often the carcinogenic HPV16 genotype (Tornesello et al. 2010). Systemic immunosuppression is associated with cervical cancer disease progression (Kosmaczewska et al. 2012). Lymphomas undergo
spontaneous remission after immunosuppression is withdrawn (Vesely et al. 2011). AIDS patients have greatly increased risks for herpes viral Kaposi’s sarcoma and for cervical cancer. Paradoxically, breast and ovarian cancer risks sometimes seem reduced (Frisch et al. 2001). Immune responses weakened or dysregulated in AIDS patients might be sources of breast cancer cell progression. Alternatively, antiretroviral therapy for AIDS may reduce breast/ovarian cancer risks but this is still unclear. Seemingly reduced breast cancer risk in transplant patients may reflect screening- (Engels et al. 2011) or sampling artifacts or increased mortality. Transplant patients with prior breast cancers had a HR > 5 times cancer specific mortality (Brattstrom et al. 2013). The immune system is essential to control EBV latent infection. Individuals with a congenital or acquired immune deficiency have increased risks for EBV-associated diseases, which vary according to the type and the severity of immunodeficiency. Lupus patients have increased risks for several malignancies. Chronic immunosuppressive drug treatment is a widely suspected risk factor.

**Hereditary immunodeficiency diseases are susceptible to specific infections because of mutations related to those found in breast cancers.** Absence of TLR3 signaling increases susceptibility to HSV-1 infection. Mutations in 3 proteins in the TLR signaling pathway have been linked to herpes simplex encephalitis (HSE) in children. Mutations in NEMO (NF-κB Essential Modulator) (Figs 1, 2) predispose primarily to infections with herpes viruses. Other primary immunodeficiencies predispose to severe infections with human papilloma viruses (Dropulic and Cohen 2011).

Most if not all endogenous retroviruses are inactivated by mutation or other mechanisms but they may become reactivated by immunodeficiency. In immunodeficient mice, active retroviruses can re-emerge through recombination to cause diseases that include cancer (Young et al. 2012). TLR3 or TLR7 deficient animals are susceptible to certain infections but remained capable of producing type I IFN in response to RNA species (Barber 2011). Dozens of studies find immunodeficient mice have increased susceptibility to spontaneous tumors. Their susceptibility to carcinogen induced cancers can depend on the immune deficit, the cancer site, and the carcinogen (Vesely et al. 2011).

**Functional consequences of mutations.** Deletion or breakage and rearrangement either eliminates or drastically changes gene function and many smaller mutations such as frame shifts can cause major losses in protein function. Here, high percentages of mutations are complete or partial gene deletions or rearrangements. For example, in breast cancer PD4103a, 161 different genes were either deleted or disrupted.
by DNA breakpoints and involved a deletion of 5-7 million bases. An additional 47 coding genes had one or more base changes, small insertions or deletions. 41 of 47 mutations in coding genes changed an amino acid in the protein product. In the remaining 6 coding mutations, transcription factor binding, gene regulation, splicing, and other interactions could all be changed. From the total of 208 deletion or coding gene mutations (161+47), only about 160 genes had usable functional information available.

In breast cancer PD4103a, virtually every one of the 160 mutations occurred in innate immunity or in metabolic pathways typically targeted by viruses (unpublished). Beyond the mutation and dysregulation in innate immunity, viruses typically disable or hijack many other host functions such as translation, calcium signaling, cell morphology, etc. Profound alterations in metabolism can accompany infection, resulting in benefits to the pathogen at host expense. Breast cancer PD4103a had these kinds of profound pathogen favoring alterations in metabolism. Many mutations in a cancer cell subclones may evolve to become fixed because they give the cancer or cancer pathogen some sort of advantage and are compatible with what is left of the immune response. However, the functional effects of most gene mutations were not directly measured in breast cancer cells. Functional effects of epigenetic changes, very large breakages and duplications (>about 7.5 Mb), mutations in many RNA encoding genes, mutations in inter- and intragenic sequences, could not be confidently evaluated. Moreover useful functional information for many genes does not exist and is urgently needed.

Conclusions and implications

Breast cancer mutations cripple the ability of the immune system to prevent or restrain cancer causing infections, to remove cells damaged by infection and to regulate inflammation. Large increases in cancer risks in immune compromised patients support the idea that accumulation of immune deficits by mutation would activate indolent cancers.

Mutations in immunity may also increase cancer risks by dysregulating responses to breast cancer with or without antigen or pathogen exposure. Mutational damage and dysregulation in the immune system and inflammatory processes also represent significant additional variables that can confound studies of viral associations with breast cancers.
The exact individual mutations potentially affecting immune responses are vastly different when one breast cancer is compared to another. Each breast cancer also has a different deficit in immunity or its regulation. Each breast cancer may differ in susceptibility to a range of carcinogenic infections. Different infections can trigger different immune responses. Thus mutational loss of a different set of genes in each breast cancer environment changes which infections are more likely to survive. Loss or damage to specific host gene products required by some infections restricts their likelihood.

Comparisons of Figs 1-2 reflect shared innate immune signal transmission pathways, so damage to shared pathways may increase the likelihood of cancer related infections in general.

Acquired defects in innate and adaptive immunity explain how many viruses have the potential to cause cancer but only in the right host. A common virus infection such as EBV is present in most people, but EBV causes cancers only rarely because most people do not have mutations that cripple the immune system genes that prevent EBV associated cancers. Genomic data does not tell which infections underlie or accompany breast cancers.

The absence of a consistent mutational profile found here stresses the need for personalized breast cancer genomic information in preventing and treating breast cancer. This is probably already feasible because of rapid precipitous declines in costs which are ongoing. Different therapeutic rationales can be envisioned but they should be based on personal breast cancer genomic information. Perhaps specific defects or dysregulation in the acute immune response can be compensated for rather than suffer further damage as a side effect of therapy. Based on genomic information, vaccines, anti-viral and/or NF-kB inhibitory drugs might have wide utility in treating or preventing recurrence of breast cancers. Several oncolytic viral therapies depend on the idea that tumor cells acquire mutations that inactivate interferon signaling. Th1 cytokines such as interferon gamma and TNF can directly induce permanent growth arrest, i.e. cellular senescence, in murine and human cancers. During mutagenesis, the inhibition of inflammasomes and products such as IL1β (*PD4115a*) and caspases (*PD4088a*) have profound effects on carcinogenesis and tumor progression (Rathinam et al. 2012).
**Tissue specificity.** The working model here is that cancer occurs in a specific tissue because immune compromise or dysregulation accompany mutational events there (e.g. from carcinogen exposures). Mutations activate infections or cause unregulated inflammation in the tissue. Thus inheriting a defect in BRCA1 or BRCA2 “breast cancer genes” should increase cancer risks not just in breast or ovary but at any site where further mutations accompany infection/inflammation. Much data supports the idea a defective BRCA gene does not cause breast cancer by itself and shows that other organs can also become susceptible, with increased risks for infection associated cancers (Friedenson 2010a, 2007). The extent of DNA damage strongly depends on exposure to particular types of mutagens such as those that cross link DNA (Levin et al. 2012). Biallelic mutations in BRCA2 or in its binding partner PALB2 are strongly associated with early childhood cancers including leukemias, medulloblastomas, Wilms tumors and neuroblastoma (Wagner et al. 2004; Reid et al. 2007), all occurring well before breast cancers.

**Role in other cancers.** Homozygous deletions in chromosome 9p include a large cluster of interferon genes and this deletion has been reported in acute lymphoblastic leukemia, gliomas and at a lower frequency in lymphomas, melanomas, lung cancers, other solid tumors. Homozygous deletions of the short arm of chromosome 9 occur in ovarian adenocarcinoma cell lines and loss of heterozygosity occurs in sporadic tumors (Chenevix-Trench et al. 1994). Breast cancer PD4088a lost this area as part of a large deletion on chromosome 9. Defensins are microbiocidal peptides within the innate immune system that are active against enveloped viruses, bacteria, and fungi. A deletion in the defensin gene DEFB127 occurs in one of the breast cancers (PD4199a) and a homozygous deletion involving defensins occurs in other cancers. These deletions suggest that the loss of immune or viral defenses occurs broadly in cancers. The arguments here are also consistent with contributions from other types of microbial infections to cancer in general. For example gastric cancer and gastric MALT lymphoma are associated with *Helicobacter pylori* infection.

**Figure Legends**
Fig. 1  Defects in signaling pathways mediated by TLR3 and TLR7 as examples of disruption of innate immunity to RNA viruses in breast cancers. Because only an outline of the pathway is shown, many interactions are indirect. Approximate points in the signaling pathway outline that show mutations, deletions or rearrangements are indicated with the name of the breast cancer in which they occur. Although different genes are damaged, there are similar impairments in overall functions so that the end result would presumably be an impaired or dysregulated immune response. Multiple defects in initial recognition of RNA viruses are indicated on the Figure. STING facilitates RIG-1 signaling (see also Fig. 2). The end steps in these pathways involve the nucleus and are largely shared in Fig. 2 but the exact genes mutated may differ. The basic pathways shown are based on Mogenson (Mogensen 2009) and Barber (Barber 2011).

Fig. 2  Defects in signaling pathways in breast cancers that disrupt or dysregulate innate immunity to DNA viruses. Defects in genes essential for viral recognition are shown next to the depiction of a DNA virus. The mechanisms for immediate recognition of viral RNA or DNA via the STING pathway are not characterized. The basic pathways shown are based on Mogenson (Mogensen 2009) and Barber (Barber 2011). The breast cancers in the gray box have mutations in genes which affect susceptibility to some viral infections (Table 2) but the genes have not been clearly linked to immunity. The figure shows potential indirect actions of other mutations such as DSTYK (a caspase inducer) and C1QTNF1 (a target of IL1β).
Tables

Table 1 Common functions essential for immune responses are disabled by mutations in a variety of genes in different breast cancer genomes (Figs 1 and 2)

<table>
<thead>
<tr>
<th>Immune function</th>
<th>Breast cancer</th>
<th>Example(s) of mutated gene (symbol) that negatively effects immune function: explanation and reference</th>
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</thead>
<tbody>
<tr>
<td><strong>RNA virus recognition</strong></td>
<td>PD3890a</td>
<td><strong>HUWE1</strong>: Novel cellular interactor of Gag-Pol through integrase binding (Yamamoto et al. 2011).</td>
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<tr>
<td></td>
<td>PD3904a</td>
<td><strong>IFIT1, IFIT2, IFIT1B, IFITS</strong>: Antiviral innate immunity relies on the recognition of microbial structures. One such structure is viral RNA that carries a triphosphate group on its 5' terminus (PPP-RNA). On the basis of this specificity and the great abundance of IFIT proteins after infection, it has been proposed that the IFIT complex antagonizes viruses by sequestering specific viral nucleic acids such as PPP-RNA (Pichlmaier et al. 2011). <strong>C163L1</strong>: scavenger receptor</td>
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<td></td>
<td>PD4006a</td>
<td><strong>FKBP8/FKBP38</strong>: T cell activation.</td>
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<td></td>
<td>PD4107a</td>
<td><strong>DROSHA</strong>: Silences endogenous retroviruses and HTLV-1 (Van Duyne et al. 2012)</td>
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<td></td>
<td>PD4109a</td>
<td><strong>APOBEC4</strong>: The human APOBEC3 gene family on chromosome 22 encodes for 7 cytidine deaminases (A3A to A3H), some with potent antiretroviral activity against retroviruses such as HTLV-1 (Rogozin et al. 2005).</td>
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<tr>
<td></td>
<td>PD4115a</td>
<td><strong>ADAR</strong>: Adenosine deaminase functions to modify viral RNA genomes.</td>
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<td></td>
<td>PD4116a</td>
<td><strong>RNASEH2C</strong>: Cleaves ribonucleotides from RNA-DNA duplexes.</td>
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<td>PD4120a</td>
<td><strong>DHX58</strong>: Pathogen recognition receptor for HCV</td>
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<td><strong>DNA virus recognition, response, inhibition</strong></td>
<td>PD3905a</td>
<td><strong>HDAC7</strong>: HDAC 7 was the most readily detected class II HDAC in Akata and Raji cells, suggesting that it may be involved in repression during EBV latency (Bryant and Farrell 2002). Toll-like receptors (TLRs) were reported to be inhibited by HDAC inhibitors (Bode et al. 2007).</td>
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<td>PD4006a</td>
<td><strong>MSN (moesin)</strong>: T-cell activation, antigen recognition. Contains a T-cell receptor responsive site. <strong>PKD2</strong>: T cell antigen receptor signaling (Navarro et al. 2012)</td>
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<td>PD4086a</td>
<td><strong>AIM2</strong>: Absent in melanoma 2 (AIM2) is a double-stranded DNA receptor, and its activation initiates an interleukin-1 beta processing inflammasome. AIM2 is implicated in host defense against several pathogens by responding to cytoplasmic double stranded DNA and double stranded DNA vaccinia virus. (de Koning et al. 2012)</td>
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<td>PD4088a</td>
<td><strong>DSCAM</strong>: pattern recognition receptor that (in drosophila) can produce thousands of isoforms via mutually exclusive alternative splicing (Ziauddin and Schneider 2012). A cloud of different immune effectors with different specificities. <strong>DSTYK</strong>: Caspase inducer.</td>
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<td>PD4103a</td>
<td><strong>GATA3</strong>: Critical early regulator of innate lymphoid cell development, thereby extending the paradigm of Gata3-dependent control of type 2 immunity to include both innate and adaptive lymphocytes (Klein Wolterink et al. 2013). GATA3 also interacts with HPV16, 18 control regions. <strong>NBN</strong>: NibrinNBS1/ p95 protein of MRE11/Rad50 complex. Complex responds to ds DNA viruses such as adenovirus. <strong>PARG</strong>: Inhibition avoids death of HSV-1 virally infected cell (Grady et al. 2012)</td>
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<td>PD4115a</td>
<td><strong>SREBP2</strong>: Host defense against viral infection (CMV model) involves IFN mediated down-regulation of sterol biosynthesis. Lowered cholesterol protects against viral infection (Blanc et al. 2011) <strong>C1QTNF1</strong>: target of IL-1 beta</td>
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<td>PD4116a</td>
<td><strong>BANF1 (BAF)</strong>: acts as a potent host defense against poxviral DNA replication in the cytoplasm (Ibrahim et al. 2011)</td>
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<td>PD4120a</td>
<td><strong>GATA3</strong>: Critical early regulator of innate lymphoid cell development, thereby extending the paradigm of Gata3-dependent control of type 2 immunity to include both innate and adaptive lymphocytes (Klein Wolterink et al. 2013). GATA3 also interacts with HPV16, 18 control regions.</td>
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<td>PD4198a</td>
<td><strong>ATM</strong>: required to control EBV and other gamma herpesviruses (Kulinski et al. 2012). ATM knockdown activates cells of the innate immune system. The DNA damage response may also participate in alerting the immune system to the presence of potentially dangerous cells (Gasser et al. 2005)</td>
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<td>PD4199a</td>
<td><strong>DEFB127</strong>: Beta defensin</td>
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<td><strong>Autophagy</strong></td>
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<td>PD3851a</td>
<td><strong>STX4</strong>: lysosome exocytosis</td>
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<td>PD3904a</td>
<td><strong>FYCO1</strong>: Autophagy protein. A subset of viruses and bacteria subvert autophagic pathways to promote their own replication (Pankiv and Johansen 2010)</td>
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<td>PD3905a</td>
<td><strong>VAMP7</strong>: Autophagy TMEM74: regulates autophagy</td>
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<td><strong>PHLDA1</strong>: Mediates and positively regulates both autophagy and apoptosis pathways</td>
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<td>PD4107a</td>
<td><strong>BCLAF1</strong>: inhibits transformation by adenovirus. Caspase-10 inhibits autophagy by cleaving the BCL2-interacting protein BCLAF1, itself a strong inducer of autophagy that acts by displacing beclin-1 from BCL2 (Lamy et al. 2013).</td>
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<td>PD4116a</td>
<td><strong>KAT5</strong>: Regulates autophagy</td>
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<td>PD4120a</td>
<td><strong>LYST</strong>: Lysosomal trafficking regulator</td>
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**Table 2** Breast cancer mutations not clearly linked to innate immunity affecting viral infections

| PD3905a | **RPS10**: Interacts with HIV-1 Nef to impair translation (Abbas et al. 2012). **SLC4A8**: antisense transcripts formed from LTR promoter elements of HERVs decrease mRNA levels of SLC4A8 (Gogvadze et al. 2009). **CCNT1**: Interacts with HIV-1 Tat protein. |
| PD4006a | **NHP21**: May be involved in regulating replication of retroviral elements (Li et al. 2012) |
| PD4116a | **VPS37B**: Functions in sorting ubiquitylated protein cargoes into multivesicular bodies, mediates receptor down regulation. Part of ESCRT-I complex required for HIV-1 budding and infectivity and virion release. |
| PD4199a | **BEND4**: Possible role in organizing viral DNA during replication or transcription. |

**References**


Friedenson B (2010b) Inflammatory processes inordinately increase tissue specific cancer risks in carriers of mutations in BRCA1, BRCA2, ATM or Fanconi anemia genes. Journal of Medicine and Medical Sciences 1 (8):356-371

Friedenson B (2010c) A theory that explains the tissue specificity of BRCA1/2 related and other hereditary cancers. Journal of Medicine and Medical Sciences 1 (8):372-384


